

Cytokines and endometrial receptivity after intracytoplasmic sperm injection — A cohort study at Islamabad

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Abstract

Objective: To evaluate the role of tumour necrosis factor alpha and interleukin-6 in conception after assisted reproductive treatment.

Methods: The longitudinal cohort study was conducted from August 2014 to May 2015 and comprised patients undergoing intracytoplasmic sperm injection at the Islamabad Clinic Serving Infertile Couples, Islamabad, Pakistan. During the procedure, endometrial thickness was measured on the day of ovulation induction and serum tumour necrosis factor alpha along with interleukin-6 were measured on the embryo transfer day. Subjects were divided into groups, with non-pregnant females in Group A and those with confirmed foetal cardiac activity in Group B and non-pregnant females were divided into two groups. The difference between the groups and the association of cytokines with endometrial thickness were measured.

Results: Of the 131 subjects, 79(60%) were in Group A and 52(40%) in Group-B. The mean level of interleukin-6 was 49.65 ± 3.04 in Group B and 104.14 ± 76.03 in Group A, with significant inverse correlation with endometrial thickness ($p < 0.001$). The level of tumour necrosis factor alpha were significantly higher in Group-B compared to Group-A ($p < 0.001$).

Conclusion: The pro-inflammatory cytokine, tumour necrosis factor alpha, takes part in preparation of endometrial lining for implantation of embryo in assisted reproductive treatment procedures.

Keywords: Endometrial thickness, Tumour necrosis factor alpha, Interleukin-6, Infertility, Intracytoplasmic sperm injection. (JPMA 68: 862; 2018)

Introduction

There is significant debate about the role of cytokines in pregnancy. These are small proteins secreted by cells of the immune system. It is a well-established fact that cytokines affect reproductive processes, such as follicular development, ovulation, fertilisation, implantation and embryonic development.¹ Cytokines concomitant with foetal and maternal interface play an imperative role in normal conception.² Interleukins (IL) are cytokines secreted by leukocytes that act on other leukocytes. IL-1 is produced by cells such as macrophages, B cells and dendritic cells and is a primary mediator of host responses. It induces prostaglandin and fibrinogen synthesis and stimulates fibroblast proliferation. IL-6 is a multifunctional cytokine, playing key roles in inflammation and T cell differentiation in adaptive immunity. IL-6 is also important for the acute phase response.³ Since it is expressed in the female reproductive tract and gestational tissues, it is believed to regulate implantation of the embryo and placental development.¹

During ovulation, follicular stimulating hormone (FSH) and luteinizing hormone (LH) lead to the production of

tumour necrosis factor alpha (TNF- α) and IL-1 β . TNF- α and IL-1 β are involved in two different pathways leading to the follicular rupture. The first is via prostaglandins which increase vascular permeability and cause the follicle to rupture, and the second is through nitrogen oxide (NO) leading directly to follicular rupture.⁴ TNF- α is a cytokine produced by monocytes and macrophages. It is necessary for regulating all the immune responses, endothelial proliferation and activation of neutrophils and other inflammatory mediators. IL-6 is also a multi-functional cytokine which works towards regulating immune responses, haematopoiesis and other defence mechanisms.⁵ Both of these mediators are also known to cause endometrial proliferation.⁶ In fact, they can be used as markers for endothelial dysfunction as well.⁵

Infertility is defined by World Health Organisation (WHO) as a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. It is a very common condition, affecting one in every four couples in developing countries according to recent WHO estimates.⁷ Although Pakistan is currently among the most populous countries of the world, and has a population growth rate of around 2%, it also has high rate of infertility which is 21.9%; 3.5% primary and 18.4% secondary.⁸ Infertility treatment procedures range from

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simple to advanced assisted reproductive treatment (ART) procedures that include in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI).⁹ The success of these procedures is dependent upon implantation of embryo in the prepared endometrial bed.

Increased levels of IL-6 have been found during pregnancy in pigs and it has been demonstrated in IVF that IL-6 will aid the division and attachment of trophoblastic cells and is hence important for implantation and pregnancy.¹⁰ However, another research has shown that decreased levels of IL-6 in samples from ovarian follicles will increase the chances of pregnancy in patients using IVF. Elevated IL-6 levels were seen in cases of unexplained infertility, recurrent miscarriage, preeclampsia and preterm delivery¹¹ as well as in endometriosis.¹² Another study conducted on Turkish women in 2015 revealed a greater concentration of IL-6 in the amniotic fluid of pre-term cases compared to full term ones.¹³

The current study, the first of its kind in Pakistan, was planned to identify which of the pro-inflammatory cytokine IL-6 or TNF takes part in preparation of endometrial bed for implantation of embryo for conception after ART.

Subjects and Method

The longitudinal cohort study was conducted from August 2014 to May 2015, and comprised women undergoing ICSI at the Islamabad Clinic Serving Infertile Couples (ICSIC). The subjects included were aged 20-40 years and had body mass index (BMI) in line with the South Asian criteria.¹⁴ Females with endocrine disorders, such as thyroid dysfunction, abnormal prolactin levels and cases of unexplained infertility, were excluded. Non-pregnant females were placed in Group A, while those confirmed foetal cardiac activity were in Group B. The protocol for ICSI was carried out on subjects after down-regulation of ovaries from the second day of their menstrual cycle followed by controlled ovarian stimulation, oocyte retrieval and embryo transfer.¹⁴

Some patients who were not able to complete all the steps of the ICSI protocol till the transfer of embryo their results were excluded.

Serum TNF- α and IL-6 were measured on the day of embryo transfer using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Pregnancy was declared positive by the presence of cardiac activity on transvaginal scan. Descriptive statistical analysis of continuous variables was presented as mean + standard deviation (SD). Statistical comparisons between the groups were performed using Mann-Whitney U test for non-parametric variables. Associations between serum levels of cytokines IL6 and TNF- α , and endometrial thickness were determined using Spearman's rank correlation considering $p < 0.001$ as significant.

Results

Of the 131 subjects, 79(60%) were in Group A and 52(40%) in Group-B. Thickness of the endothelial lining was significantly higher in Group B compared to Group A ($p < 0.001$). The mean level of IL-6 was 49.65 ± 3.04 in Group

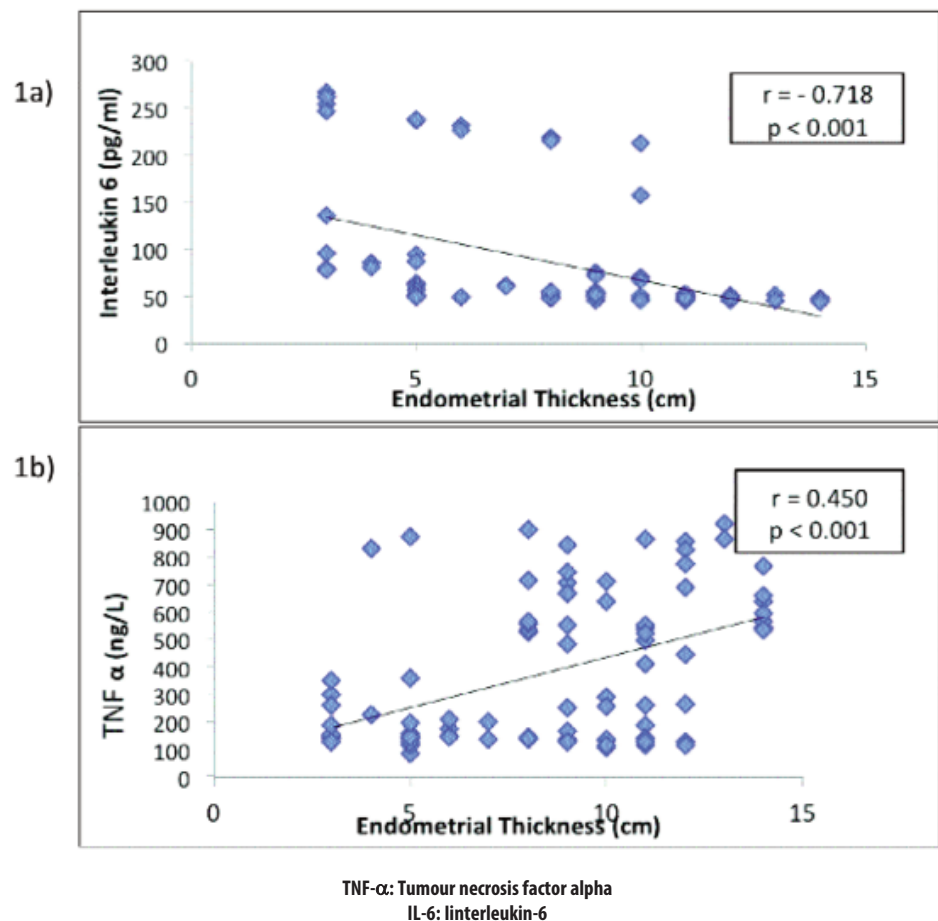


Figure-1: a) Correlation between IL-6 and Endometrial thickness. b) Correlation between TNF α and Endometrial thickness.

Table: Clinical and Biochemical data of Study Groups.

Variables	Group A (NP) (n= 79) Median $\pm$ SD	Group B (CP) (n=52) Median $\pm$ SD	P-value
Age (year)	32.00 $\pm$ 4.90	33.30 $\pm$ 5.31	0.731
BMI (kg/m ²)	23.00 $\pm$ 3.56	23.00 $\pm$ 3.38	0.054
Pre-ovulatory Follicle Count	7.0 $\pm$ 1.48	8.40 $\pm$ 1.86	<0.001
Endometrial lining (mm)	6.66 $\pm$ 2.83	11.1 $\pm$ 2.01	<0.001
Interleukin-6 (pg/ml)	104.14 $\pm$ 76.03	49.65 $\pm$ 3.04	<0.001
Tumour necrosis factor (TNF) (ng/L)	147.48 $\pm$ 59.93	596.57 $\pm$ 153.95	<0.001

NP: Non-pregnant

CP: Clinically pregnant

BMI: Body mass index

TNF: Tumour necrosis factor.

B and 104.14 ± 76.03 in Group A ($p < 0.001$), however levels of TNF- α were significantly higher in Group B compared to Group A ($p < 0.001$) (Table).

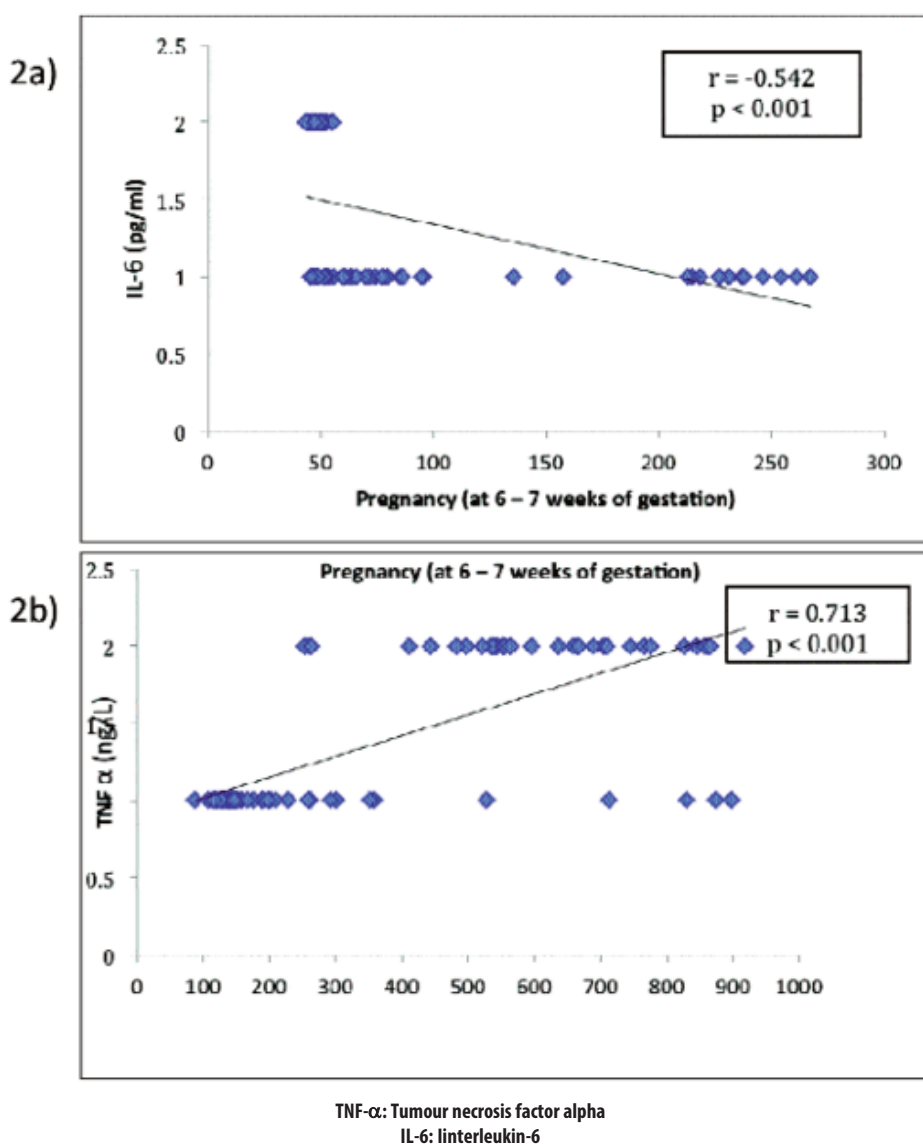
The serum concentration of IL6 was associated with endometrial thickness ($p < 0.001$) and pregnancy ($p < 0.001$), but TNF was in positive association with endometrial thickness ($p < 0.001$) and pregnancy ($p < 0.001$) (Figures 1-2).

Discussion

Pregnancy is characterised by an increase in anti-inflammatory and pro-inflammatory markers compared to a non-pregnant state, whereby early and late pregnancy states are characterised by inflammatory changes and mid-

pregnancy is characterised by anti-inflammatory changes.¹⁵ Human pregnancy has two interfaces; the first one being the interaction of maternal inflammatory cells with cytotrophoblasts and second one being the interaction of maternal inflammatory cells with syncytiotrophoblasts. Thus at these interface levels, leukocytosis, complement activation and endothelial activation are very prominent which results in synthesis of proinflammatory cytokines such as IL6 and TNF- α .¹⁶

There have been a few studies done on this topic previously which reported that the levels of TNF- α and IL-6 both increased in pregnancy and continued to increase in preeclampsia and as it progressed.¹⁷ We observed low levels of IL-6 which is contradictory to another study which suggested that IL-6 increases significantly in pregnancy.¹⁶ In 2007 a study reported that the levels of both IL-6 and TNF- α are higher in pregnancy compared to non-pregnant females and they continue to increase throughout the pregnancy.¹⁸ However, there are some studies that differ from the aforementioned results. A couple of researches in 1998 and 2003 showed that there was no significant difference in the levels

**Figure-2:** a) Correlation between IL6 and Pregnancy. b) Correlation between TNF α and Pregnancy.

of either TNF- α or IL-6 between the pregnant and non-pregnant groups.^{19,20} In contrast, our study showed a rise in TNF- α , being in line with other studies but it showed a significant decrease in levels of IL-6 in pregnant females.^{5,6}

The endometrial lining undergoes changes during the menstrual cycle. It becomes vascularised, thicker and more conducive to implantation during the implantation window period. Hence, endometrial thickness may be a good indicator of outcome in fertility treatments.²¹ Endometrial change involves the interleukins. IL-1 β can cause angiogenesis and, hence, lead to vascularisation and thickening of the endometrial lining.²² Although, evidence shows that TNF- α and IL-6 are needed for endometrial proliferation,²³ our study suggests otherwise for IL-6. Many other maternal factors would be needed to be taken into consideration before making any conclusion. These factors might include maternal health condition before and during pregnancy, multiparity, and other cytokine levels during pregnancy such as leptin, interferon gamma etc.

A study of human granulosa tumour cell lines suggests that IL-6 may impair oestradiol synthesis by the granulosa cells of the ovary. It was shown that oestradiol secretion is considerably inhibited by addition of IL-6.²⁴ This shows that IL-6 has an important role to play in folliculogenesis and proliferation of endometrium.

Other than this source, IL6 is primarily produced by the macrophages, but also by the decidua and trophoblastic cells, thus is believed to be directly involved in abortion.²⁵ This shows that IL-6 may in fact decrease how receptive the endometrium is to an implantation.²⁶ High levels of pro-inflammatory cytokines IL-6, IL-8, and TNF- α have been found to characterise early embryo implantation. They are secreted by either endometrial cells or immune cells that are recruited to the site of embryo implantation.^{16,17} As in any inflammation, the endometrial inflammation is also accompanied by the induction of adhesion molecules that play an important role in establishing an embryo implantation environment.⁶ Besides this, the role of IL-6 in abnormal immune activation in preeclamptic females has also been proved.²⁷

Study of only two pro-inflammatory cytokines is a limitation of this study. Also, it is a single-centre study which needs to be elaborated and strengthened by further studies on molecular basis.

Conclusion

Pro-inflammatory cytokines assisted as well as inhibited the implantation of embryo after ICSI. TNF- α facilitated in the implantation by increasing the endometrial receptivity, whereas IL-6 inhibited the implantation, hence reducing the chances of conception after ICSI. The estimation of IL-6 can be used as a marker to assess endometrial receptivity at the time of implantation and during gestation, especially in high-risk cases where further intervention may be required.

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Conflict of Interest: None.

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